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Citation for the original published paper (version of record):

Gardner, A., Jiang, M., Ellsworth, D S., MacKenzie, A R., Pritchard, J. et al. (2023) Optimal stomatal theory predicts CO_2 responses of stomatal conductance in both gymnosperm and angiosperm trees
New Phytologist, 237(4): 1229-1241
https://doi.org/10.1111/nph.18618

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New Phytologist

Research

Optimal stomatal theory predicts CO_2 responses of stomatal conductance in both gymnosperm and angiosperm trees

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Received: *18 July 2022*
Accepted: *17 October 2022*

New Phytologist (2023) **237:** 1229–1241
doi: 10.1111/nph.18618

Key words: climate change, deciduous, evergreen, free-air CO_2 enrichment, photosynthesis, water-use efficiency.

Summary

- Optimal stomatal theory predicts that stomata operate to maximise photosynthesis (A_{net}) and minimise transpirational water loss to achieve optimal intrinsic water-use efficiency (iWUE). We tested whether this theory can predict stomatal responses to elevated atmospheric CO_2 (eCO_2), and whether it can capture differences in responsiveness among woody plant functional types (PFTs).
- We conducted a meta-analysis of tree studies of the effect of eCO_2 on iWUE and its components A_{net} and stomatal conductance (g_s). We compared three PFTs, using the unified stomatal optimisation (USO) model to account for confounding effects of leaf–air vapour pressure difference (D). We expected smaller g_s, but greater A_{net}, responses to eCO_2 in gymnosperms compared with angiosperm PFTs.
- We found that iWUE increased in proportion to increasing eCO_2 in all PFTs, and that increases in A_{net} had stronger effects than reductions in g_s. The USO model correctly captured stomatal behaviour with eCO_2 across most datasets. The chief difference among PFTs was a lower stomatal slope parameter (g_1) for the gymnosperm, compared with angiosperm, species.
- Land surface models can use the USO model to describe stomatal behaviour under changing atmospheric CO_2 conditions.

Introduction

Stomata are small pores in vascular plant leaves that open and close to allow the passive exchange of gases between the atmosphere and a plant's internal surfaces. It has long been recognised that stomatal behaviour represents a fundamental compromise between carbon dioxide (CO_2) diffusion into the plant and simultaneous water loss (Cowan, 1982; Jones & Sutherland, 1991) such that maintaining stomatal opening, to allow CO_2 to diffuse into the sub-stomatal cavity, implies substantial diffusive water loss via transpiration. Stomatal opening is sensitive to both endogenous and external drivers (Brodribb *et al.*, 2009). Predicting stomatal responses to the environment is crucial as stomatal aperture determines both photosynthetic carbon gain and the rate of water loss in plants (Cowan & Farquhar, 1977), thus affecting intrinsic water-use efficiency (iWUE; the ratio of net photosynthetic CO_2 assimilation, A_{net}, to stomatal conductance to water vapour, g_s). Understanding the magnitude and drivers of iWUE in our changing climate is key to modelling and predicting global carbon and water cycles under a future climate (Walker *et al.*, 2020).

Atmospheric CO_2 concentration (C_a) directly affects stomatal aperture (Morison, 1987; Franks *et al.*, 2012). For example, Medlyn *et al.* (2001) reported that g_s was reduced by an average

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of 21% in European forest trees with exposure to elevated CO_2 (eCO_2; +350 μmol mol^{-1}) across four types of CO_2 exposure facilities. Similarly, Ainsworth & Rogers (2007) reported g_s was reduced by an average of 19% in response to eCO_2 (+208 μmol mol^{-1}) in tree species growing in free-air CO_2 enrichment (FACE) experiments. This reduction in water use with rising CO_2 could have large-scale consequences, including increased resilience of vegetation to drought (Jiang *et al.*, 2021) and global increases in streamflow (Gedney *et al.*, 2006) contributing to increased greening in water-limited regions (Keenan & Williams, 2018).

However, not all species respond to eCO_2 to the same extent (Saxe *et al.*, 1998), and there are species-specific stomatal responses to eCO_2 that are apparent among eCO_2 experiments (Saxe *et al.*, 1998; Medlyn *et al.*, 2001). For tree species, it has been observed that eCO_2-induced reductions in g_s are small or absent in gymnosperm species especially when compared with those in angiosperm species (Saxe *et al.*, 1998; Medlyn *et al.*, 2001; Klein & Ramon, 2019), which suggests that stomatal sensitivity to eCO_2 may vary with phylogeny (Brodribb *et al.*, 2009; Lammertsma *et al.*, 2011; Hasper *et al.*, 2017). For example, in a meta-analysis of field-based chamber experiments on forest trees, Medlyn *et al.* (2001) found that the C_a response of g_s was significantly stronger in deciduous broadleaved angiosperm trees than in coniferous species (all gymnosperms), in the set of experiments examined, although they also noted that this difference was confounded with tree age. Recently, a meta-analysis conducted by Klein & Ramon (2019) also found a significant difference in stomatal responses to eCO_2 between gymnosperm and angiosperm species. In addition to g_s responses in isolation, there are also indications that iWUE (A_{net}/g_s) may respond differently to rising CO_2 between angiosperm and gymnosperm species. For example, it has been suggested that iWUE in gymnosperms may have a stronger positive response to eCO_2, compared with angiosperms, as a result of differences in leaf structure (Niinemets *et al.*, 2011).

If there are indeed differences between angiosperm and gymnosperm, or more specifically between plant functional types (PFTs), in their stomatal responses to rising C_a, then it is important to capture these differences in vegetation models. Many vegetation models still employ a stomatal model of the form proposed by Ball *et al.* (1987), which was shown by Medlyn *et al.* (2011) to be consistent with the optimal stomatal theory put forward by Cowan & Farquhar (1977). This theory hypothesises that stomata behave in a manner that maximises CO_2 fixation through photosynthesis while minimising water loss via transpiration, by maximising the expression, $A_{net} - \lambda E$, where A_{net} is photosynthesis (μmol CO_2 m^{-2} leaf s^{-1}), E is transpiration (mol H_2O m^{-2} leaf s^{-1}) and λ (μmol CO_2 mol^{-1} H_2O) represents the marginal 'carbon cost of water use' to the plant (cf. Cowan & Farquhar, 1977). That is, the optimal stomatal conductance is that which maximises

$$\max(A_{net} - \lambda E) \qquad \text{Eqn 1}$$

Medlyn *et al.* (2011) showed that this theory, when coupled to a biochemical model of photosynthesis (Farquhar & von Caemmerer, 1982), results in an expression for g_s (mol H_2O m^{-2} leaf s^{-1}) that can be approximated as follows, also known as the unified stomatal optimisation (USO) model:

$$g_s \approx 1.6 \left(1 + \frac{g_1}{\sqrt{D}}\right) A_{net}/C_a \qquad \text{Eqn 2}$$

where g_1 ($kPa^{0.5}$) is a collection of constant terms including an inverse dependence on λ, D is the leaf–air vapour pressure difference (kPa), 1.6 is the apparent ratio of the diffusion coefficients of water vapour and CO_2, and g_s and A_{net} are the functions of D and air temperature, T_{air}, as well as other physiological parameters. Note that here λ is defined according to Hari *et al.* (1986), and is the inverse of the original definition in Cowan & Farquhar (1977) and Medlyn *et al.* (2011). In addition, the empirical intercept term, g_0, added by Medlyn *et al.* (2011) is unnecessary here and is omitted for simplicity. As the marginal cost of water λ is unknown, the parameter g_1 is obtained in practice by fitting to data. Assuming the model parameter g_1 is constant, and under environmental conditions producing constant D, the USO predicts that the A_{net}/g_s ratio, that is the intrinsic WUE (iWUE = A_{net}/g_s) should be proportional to the atmospheric CO_2 concentration (Barton *et al.*, 2012). This model has been used to predict stomatal responses successfully in several CO_2 manipulation experiments (e.g. Barton *et al.*, 2012; De Kauwe *et al.*, 2013; Gimeno *et al.*, 2016).

There are several ways in which a differential response of g_s to C_a could be captured by the USO model. First, there may be a difference in the T_{air} or D at which measurements were made. The relative responsiveness of A_{net} and g_s can vary within an experiment depending on T_{air} or D. For example, A_{net} is more responsive to eCO_2 at high leaf temperatures (Long, 1991). Additional complications may occur if D differs between treatments. Barton *et al.* (2012) showed that iWUE was proportional to eCO_2 in *Eucalyptus saligna*, once treatment differences in D were taken into account. They also showed that the responsiveness of g_s to eCO_2 decreased at high D, when high T_{air} meant that photosynthesis was more responsive for biochemical reasons (Long, 1991). These mechanisms will be captured in the USO model, which incorporates an effect of D, especially when coupled to a photosynthetic biochemistry model that captures interactions with T_{air} and C_a (e.g. Duursma, 2015).

Second, a substantial increase in iWUE in response to eCO_2 can occur as a strong increase in A_{net}, with a marginal reduction in g_s, or a marginal increase in A_{net} and a strong reduction in g_s. Photosynthetic responses may be stronger in species that have a low g_1 and consequently a low intercellular CO_2. There is strong evidence that gymnosperm trees have a lower g_1 than angiosperm trees (e.g. Lin *et al.*, 2015), so this is a plausible mechanism for species or PFT differences. In addition, photosynthetic responses to eCO_2 are expected to be larger in species with thicker or denser leaves because thicker leaves have greater diffusive limitation of the transport of CO_2 to the sites of carboxylation, and so respond more positively to eCO_2 than species with thinner leaves (Niinemets *et al.*, 2011). This mechanism would explain a

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difference between angiosperms and gymnosperm PFTs, but would also suggest a difference in responsiveness between evergreen and deciduous angiosperms, since evergreen species typically have thicker leaves (Wright *et al.*, 2004; Poorter *et al.*, 2009; Kattge *et al.*, 2011). These mechanisms would be captured in the model by specifying a different value of g_1 across species or PFTs.

Finally, it is also possible that the USO model does not accurately capture responses to eCO_2 in gymnosperm PFTs unless it is assumed that the slope parameter g_1 changes with treatment (i.e. g_1 is a function of C_a). For example, Uddling & Wallin (2012) found no decrease in g_s at eCO_2 in *Picea abies*. They found that the response of iWUE was considerably less than predicted from the optimal stomatal theory (Eqn 2) and that the slope parameter g_1 increased from 1.45 to 2.44 ($kPa^{0.5}$) between aCO_2 and eCO_2 treatments. This mechanism could potentially be captured in the model by specifying g_1 as a function of C_a.

In this paper, we examined the effect of eCO_2 on iWUE, g_s and A_{net} responses across a diverse set of field-based forest and tree plantation experiments of deciduous and evergreen angiosperms and evergreen gymnosperms. We focused on experiments with sufficient data available to evaluate the USO model (Eqn 2). A series of FACE and whole-tree chamber (WTC) experiments over the last two decades have contributed detailed datasets across a variety of species that include larger and older trees and ecosystem-scale experiments which provide the opportunity to re-examine stomatal responses to eCO_2 (Ellsworth, 1999; Gunderson *et al.*, 2002; Bernacchi *et al.*, 2003; Uddling *et al.*, 2009; Bader *et al.*, 2010, 2016; Gimeno *et al.*, 2016). We used these data to test whether there are differences in responsiveness of g_s and iWUE between different PFTs, and to utilise the optimal stomatal theory to explore the reasons for these differences. We also use the data to test a number of other hypotheses arising from optimal stomatal theory. For example, the theory implies that the parameter g_1 will be lowest in plants with a relatively high marginal carbon cost of water and therefore low hydraulic conductance (Medlyn *et al.*, 2011). Previous research shows that branch-level hydraulic conductance is relatively low in gymnosperms (Becker *et al.*, 1999), compared with angiosperms, and may decline with tree age (Hubbard *et al.*, 1999), suggesting that gymnosperms and older trees are likely to have a relatively high marginal carbon cost of water.

We hypothesised that (1) following USO, iWUE would increase in proportion to the CO_2 increase similarly across all species; (2) on average, g_s would decrease and A_{net} would increase in response to eCO_2, with A_{net} being the principal driver for increased iWUE; (3) the magnitude of change in A_{net} to eCO_2 would depend on D, with the response of A_{net} to eCO_2 larger at high D; and (4) the magnitude of change in both A_{net} and g_s to eCO_2 would depend on PFT, with A_{net} being more responsive and g_s being less responsive in evergreen gymnosperms, when compared with angiosperm PFT. We separate angiosperms into evergreen and deciduous groups to further test for differences in responsiveness associated with leaf morphology. We anticipated that differences in stomatal responses between PFTs (evergreen and deciduous angiosperm and evergreen gymnosperm) could be captured through the USO model, which accounts for variability in D and can be parameterised with different values of g_1 for different PFTs. We predicted that the optimal stomatal theory predicts that g_1 values will be lower in gymnosperms (Lloyd & Farquhar, 1994; Medlyn *et al.*, 2011; Lin *et al.*, 2015), compared with angiosperms, and older trees, compared with younger trees.

Materials and Methods

Datasets

To fit the optimal stomatal model, we need detailed individual leaf gas exchange measurements with supporting environmental data such as photosynthetic photon flux density (PPFD), leaf temperature (T_{leaf}), T_{air} and D. Some datasets, particularly those using open top chambers or branch bag (BB) approaches, were also used by Medlyn *et al.* (2001). We added available datasets from more recent major field-based eCO_2 experiments on field-grown plantation or forest trees, which largely used FACE or WTC approaches. Overall, 20 distinct datasets were obtained, covering 17 tree species at 16 separate field-based eCO_2 experiments across 13 study sites (Table 1).

The experiments differed in a number of ways, including but not limited to site climate, CO_2 fumigation treatment and experimental duration (Tables 1, 2). We selected data for which CO_2 concentration was the only treatment variable, and we excluded data collected under additional manipulative treatments such as nutrient, drought, temperature or ozone treatments. There were two factors common to all experiments: the experiments were conducted on freely rooted trees and all studies included at least two growing seasons. Medlyn *et al.* (2001) demonstrated high variability in stomatal responses in experiments running for less than half a year, particularly for those in pots. The one exception to this was the second Swiss Canopy Crane experiment, with *P. abies*, for which suitable data were only available from the initial 2 months after the start of CO_2 fumigation (Table 1). All experimental approaches were given equal weight in the meta-analysis.

The 17 study species were split into three PFTs: evergreen gymnosperm forest (EGF; $n = 3$), evergreen angiosperm forest (EAF; $n = 3$) and deciduous angiosperm forest (DAF; $n = 11$). The EAF PFT only included species of the *Eucalyptus* genus. In total, 3661 leaf gas exchange measurements were obtained. In each experiment, plants were grown at two atmospheric CO_2 concentrations which, across the experiments, had means of 383 and 628 μmol mol^{-1}, respectively (Table 2; Fig. S1). The experiments took place between 1993 and 2019 (or, in global average ambient C_a terms, from 357 to 411 μmol mol^{-1}), with the experimental duration ranging from 1 to 12 yr. All datasets consisted of either instantaneous spot measurements of A_{net} and g_s or CO_2 response curves (i.e. A-C_i curves), from which the initial point at treatment CO_2 level was taken. Measurements were made using several gas exchange systems, with the Li-6400 as the most common instrument (Li-Cor Inc., Lincoln, NE, USA). The datasets were split into three discrete categories of tree age: aged (> 80 yr old; $n = 5$), mature (10 < age < 80 yr old; $n = 6$) and sapling (< 10 yr old; $n = 9$) in addition to two categories of water condition (i.e. irrigated ($n = 6$) or non-irrigated ($n = 14$)).

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New Phytologist (2023) 237: 1229–1241
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Table 1 Details of experiments from which data were obtained.

Name	Species	PFT	Year(s) of sampling	Experimental set up	Longitude	Latitude	MAT (°C)	Plant age	Plant age category	Water condition category	Year(s) of CO_2 exposure	Number of replicates	Primary reference
Glencorse_1, UK*	*Picea sitchensis*	EGF	1993	BB	–3.12°W	55.31°N	8.3	16	Mature	Not irrigated	4	6	Barton & Jarvis (1999)
Flakaliden_1, Sweden*	*Picea abies*	EGF	1994–1995	BB	19.27°E	64.07°N	2.4	29	Mature	Not irrigated	4	6	Roberntz & Stockfors (1998)
Duke FACE, USA (NC)	*Pinus taeda*	EGF	1998–2006	FACE	–79.09°W	35.97°N	14.8	15–23	Mature	Not irrigated	4–12	3	Ellsworth *et al*. (2012)
Swiss Canopy Crane_2, Switzerland	*Picea abies*	EGF	2009	webFACE	7.30°E	47.28°N	11.7	100	Old	Not irrigated	2 months	5	Bader *et al*. (2016)
Flakaliden_2, Sweden	*Picea abies*	EGF	2003–2004	WTC	19.27°E	64.07°N	2.4	45	Mature	Not irrigated	> 2	3	Uddling & Wallin (2012)
Glencorse_2, UK*	*Betula pendula*	DAF	1994	OTC	–3.12°W	55.31°N	8.3	0–4	Sapling	Not irrigated	4	6	Rey & Jarvis (1997)
Gribskov, Denmark*	*Fagus sylvatica*	DAF	1996	BB	12.16°E	55.59°N	8.8	36	Mature	Not irrigated	2	4	Sigurdsson *et al*. (2002)
ORNL, USA (TN)	*Liquidambar styraciflua*	DAF	1999, 2008–2009	FACE	–84.20°W	35.54°N	14.8	20	Mature	Not irrigated	1,9 and 10	2	Warren *et al*. (2015)
Swiss Canopy Crane_1, Switzerland	*Quercus petraea*	DAF	2001	webFACE	7.30°E	47.28°N	11.7	100	Old	Not irrigated	1	3	Bader *et al*. (2010)
	Fagus sylvatica	DAF										3	
BIFoR FACE, UK	*Quercus robur*	DAF	2019	FACE	–2.03°W	52.80°N	9	175	Old	Not irrigated	4	3	Gardner *et al*. (2021)
Rhinelander, USA (WI)	*Betula papyrifera*	DAF	2005–2006	FACE	–89.05°W	45.06°N	6	8–9	Sapling	Not irrigated	7–8	3	Uddling *et al*. (2009)
	Populus tremuloides	DAF										3	
POPFACE, Italy	*Populus nigra*	DAF	2000	FACE	11.48°E	42.22°N	14	1	Sapling	Irrigated	1	6	Bernacchi *et al*. (2003)
	Populus euramericana	DAF										12	
	Populus alba	DAF										12	
Gunnarsholt, Iceland	*Populus trichocarpa*	DAF	1996	WTC	–20.13°W	6351°N	5.2	4	Sapling	Irrigated	3	4	Sigurdsson *et al*. (2002)
Richmond_1, Australia	*Eucalyptus saligna*	EAF	2008–2009	WTC	150.44°E	–33.36°S	17.2	2	Sapling	Irrigated	2	3	Barton *et al*. (2012)
Richmond_2, Australia	*Eucalyptus globulus*	EAF	2010–2011	WTC	150.44°E	–33.36°S	17.2	15 months	Sapling	Irrigated	15 months	3	Quentin *et al*. (2015)
EucFACE, Australia	*Eucalyptus tereticornis*	EAF	2013	FACE	150.44°E	–33.37°S	17.2	80	Old	Not irrigated	6 months	3	Gimeno *et al*. (2016)

DAF, deciduous angiosperm; EAF, evergreen angiosperm; EGF, evergreen gymnosperm; PFT, plant functional type. Experimental set-up: branch bag (BB), open top chamber (OTC), whole-tree chamber (WTC) and free-air CO_2 enrichment (FACE). Mean annual temperature (MAT; °C). Plant age and length of CO_2 exposure are given in years unless otherwise stated. Number of replicates refers to the number of 'true' replicates in each study (number of trees in BB, OTC, WTC and webFACE studies; number of rings in FACE studies).
*, Experiments for which data were included in Medlyn *et al*. (2001).

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Table 2 Measurement details.

Experiment name	Species	Equipment	Measurement type	Total no. data points	Measurement conditions				
					PPFD (μmol m^{-2} s^{-1})	Temperature (°C)	*D* (kPa)	Mean CO_2 (μmol mol^{-1})	
								aCO_2	eCO_2
Glencorse_1, UK*	*Picea sitchensis*	ADC LCA3 + light source	*A*-C_i	116	800	18–35		326	657
Flakaliden_1, Sweden*	*Picea abies*	LI-Cor 6200 + light source	*A*-C_i	63	1000	5–24 (T_{air})	0–1.1	343	703
Duke FACE, USA (NC)	*Pinus taeda*	Li-Cor 6400 + light source	*A*-C_i and spot measurements	152	1500	10–31	0.69–2.95	364	563
SCC_2, Switzerland	*Picea abies*	Li-Cor 6400XT + light source	*A*-C_i	83	663–1804	–	0.4–7.1	369	527
Flakaliden_2, Sweden	*Picea abies*	Custom made gas exchange system	Automated cuvette	2010	1000–1995	8.5–37	0.26–6.3	342	665
Glencorse_2, UK*	*Betula pendula*	Li-Cor 6200 + home made light source	Spot measurements	135	1200	18.4–32.8	0.8–3.0	344	701
Gribskov	*Fagus sylvatica*	Ciras-1 + light source	Spot measurements	16	1000	25.6–29	1.7–3.2	347	700
ORNL, USA (TN)	*Liquidambar styraciflua*	Li-Cor 6400XT	*A*-C_i	115	1800	–	0.8–3.4	372	554
SCC_1, Switzerland	*Quercus petraea*	Li-Cor 6400 + light source	Spot measurements	68	1000	23–27	0.7–1.86	380	550
	Fagus sylvatica			98	1000	23–28	0.5–1.7	380	550
BIFoR FACE, UK	*Quercus robur*	Li-Cor 6800 + light source	*A*-C_i	43	1500	24–28	1.07–2.3	383	527
Rhinelander, USA (WI)	*Betula papyrifera*	Li-Cor 6400 + light source	Spot measurements	75	1800	23–38	0.9–3.8	365	557
	Populus tremuloides			74	1800	22–37	0.59–4.1	366	560
POPFACE, Italy	*Populus nigra*	Li-Cor 6400	Spot measurements	35	870–2000	21–34	0.84–2.88	368	565
	Populus euramericana			55	847–2000	21–35	0.57–2.7	355	562
	Populus alba			51	834–2000	25–35	0.68–3.4	356	566
Gunnarsholt	*Populus trichocarpa*	Li-Cor 6200 or Ciras-1	Spot measurements	44	1200	6–26	0.38–1.4	352	702
Richmond_1, Australia	*Eucalyptus saligna*	Li-Cor 6400XT + light source	Spot measurements	197	1000–1800	15–43	0.5–6.47	384	625
Richmond_2, Australia	*Eucalyptus globulus*	Li-Cor 6400XT + light source	Spot measurements	103	1800	19–39	0.8–4.9	390	627
EucFACE, Australia	*Eucalyptus tereticornis*	Li-Cor 6400XT + light source	Spot measurements	132	1800	18–39	0.7–6.2	398	544

'Experiment name' refers to the facility and location of the dataset. 'Equipment' refers to the gas exchange equipment used to collect the data. 'Measurement type' refers to the measurements conducted in each dataset. PPFD refers to photosynthetic photon flux density. Temperature refers to leaf temperature (T_{leaf}) unless otherwise stated and is shown as the range at which measurements were taken. *D* refers to the leaf–air vapour pressure difference and is shown as the range at which measurements were taken. Mean CO_2 refers to the mean CO_2 treatment in either the ambient CO_2 (aCO_2) or elevated CO_2 (eCO_2) plots at each site.
*, Experiments for which data were included in Medlyn *et al*. (2001).

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Details of each experiment and measurement conditions are given in Tables 1, 2 respectively. Further information on each experiment can also be found in the individual references provided in Table 1.

The datasets were collected across prevailing environmental conditions which included a range of light (PPFD) and D conditions. As our hypotheses centre on D and not PPFD, we filtered out data measured at PPFD < 800 μmol m^{-2} s^{-1}. The majority of our datasets were measured at a controlled value of saturating light; only two studies used variable light and were therefore subject to filtering (Bernacchi *et al.*, 2003; Uddling *et al.*, 2009). In the one dataset where D was not reported (Roberntz & Stockfors, 1998), we calculated a lower bound to D using data on air temperature (T_{air}) and relative humidity. In all other cases, leaf-to-air-D was used.

Data analysis

We first conducted a standard meta-analysis of the data. We then separated data into D bins to conduct a second meta-analysis accounting for differences in D among treatments and experiments. Finally, we fit the USO model to the data and explored model fits and fitted parameters.

For each experiment, we compiled the A_{net}, g_s, iWUE, C_a, D and T_{leaf} data and calculated the means, standard deviation and sample size under elevated and ambient (control) CO_2 plots using the experiment replicates stated by each individual study. We calculated overall effects of eCO_2 in a weighted, mixed-effects model using the rma.mv function from the R package METAFOR (Viechtbauer, 2010). Plant functional type was a fixed factor and the potential dependency of studies within the same site (e.g. different species, different treatments) was accounted for by including study site and species as random effects. All response variables were log-transformed before analysis so that statistics investigate a potential power law relation. To quantify and standardise the CO_2 response ratios across varying ranges of CO_2 manipulation, we report data as a relativised value of CO_2 responses, that is a CO_2 normalised response ratio (r_c) as follows:

$$r_c = \frac{\log_e\left(\frac{X_e}{X_a}\right)}{\log_e\left(\frac{C_e}{C_a}\right)} \quad \text{Eqn 3}$$

where X_e and X_a represent the values of the response variables (iWUE, g_s and A_{net}) at ambient or lower CO_2 (C_a) and elevated or higher CO_2 (C_e) respectively. A value of $r_c = 1$ represents direct (linear) proportionality between a variable's CO_2 response and the change in CO_2. Values of $r_c \neq 1$ indicate sub- or super-linear responses, depending on whether $r_c < 1$ or $r_c > 1$ respectively. As $r_c \to 0$, the treatment effect approaches zero (i.e. $X_e = X_a$). We report uncertainties as 95% confidence intervals (CI) on r_c as calculated by the rma.rv function and significant responses were recognised if the CI did not overlap zero. Effect size measurements from individual studies in the meta-analysis were weighted by the inverse of the variance (Eqn 4).

$$\text{var} = \frac{SD^2_{C_e}}{n_{C_e}\overline{X}_{C_e}^{\,2}} + \frac{SD^2_{C_a}}{n_{C_a}\overline{X}_{C_a}^{\,2}} \quad \text{Eqn 4}$$

where SD represents the standard deviation, n represents the number of experimental replicates, $\overline{X}$ represents the mean, and C_e and C_a represent elevated and ambient C_a. To examine the effect of D on the response ratios, we binned observations into 0.5 kPa bins of D for each species and dataset within measurement campaigns. The bin width was chosen such that it allowed sufficient data per bin ($n = 3$) to calculate a mean response within each D bin without losing resolution of the D response. For this analysis, we only included D bins that overlap among experiments (0.75–2.75 kPa). We constructed mixed-effects models for each PFT with sensitivity to CO_2 as the dependent variable. The D bin was taken as a fixed effect and study site and species as random effects. We report the statistically significant predictors.

We fitted the USO model (Eqn 2) to the measurements of gas exchange for each tree species and dataset within each measurement campaign. Note that we here do not use an intercept (g_0) in the model as it can bias the g_1 value (Duursma *et al.*, 2019). We obtained R^2 values for each study site and species by comparing the fitted predicted values to the observed values. To test for significant differences in g_1 (Eqn 2) between CO_2 treatments, we fitted the model using a dummy variable for treatment level. To test for significant differences in g_1 among PFT, tree age and water condition (as defined in Table 1), we constructed mixed-effects models using the rma.mv function from the R package METAFOR (Viechtbauer, 2010). Plant functional type, tree age category and water condition category were fixed effects and study site and species were random effects.

All analyses in this study were conducted using R v.4.0.2 (R Core Team, 2022). The datasets are publicly available (see 'Data availability').

Results

Mean responses to eCO_2

We found that eCO_2 significantly increased plant iWUE for all vegetation types ($P < 0.001$, Fig. 1a–c showing mean ± 95% CIs). We could not disprove the hypothesis that the magnitude of the iWUE response is proportional to increase in CO_2; the mean r_c was not significantly different from 1 (overall mean r_c = 0.89 (95% CI: 0.79, 1.01)). This proportionality did not differ significantly among vegetation types (Table 3, 95% CIs = 0.78–1.27 for EAF, 0.73–1.02 for DAF, and 0.68–1.08 for EGF), supporting the prediction of the USO model that iWUE increases in proportion to the CO_2 increase, across all PFTs.

Across the range of eCO_2 experiments we analysed, stomatal response to eCO_2 had an overall mean response that was not significantly different from zero (mean $r_c = -0.09$ (95% CI: −0.26, 0.08)), indicating no mean reduction in g_s. Looking at the PFTs individually, there was a significant reduction in g_s with eCO_2 in EAF trees ($P < 0.05$, mean $r_c = -0.46$, (95% CI: −0.85,

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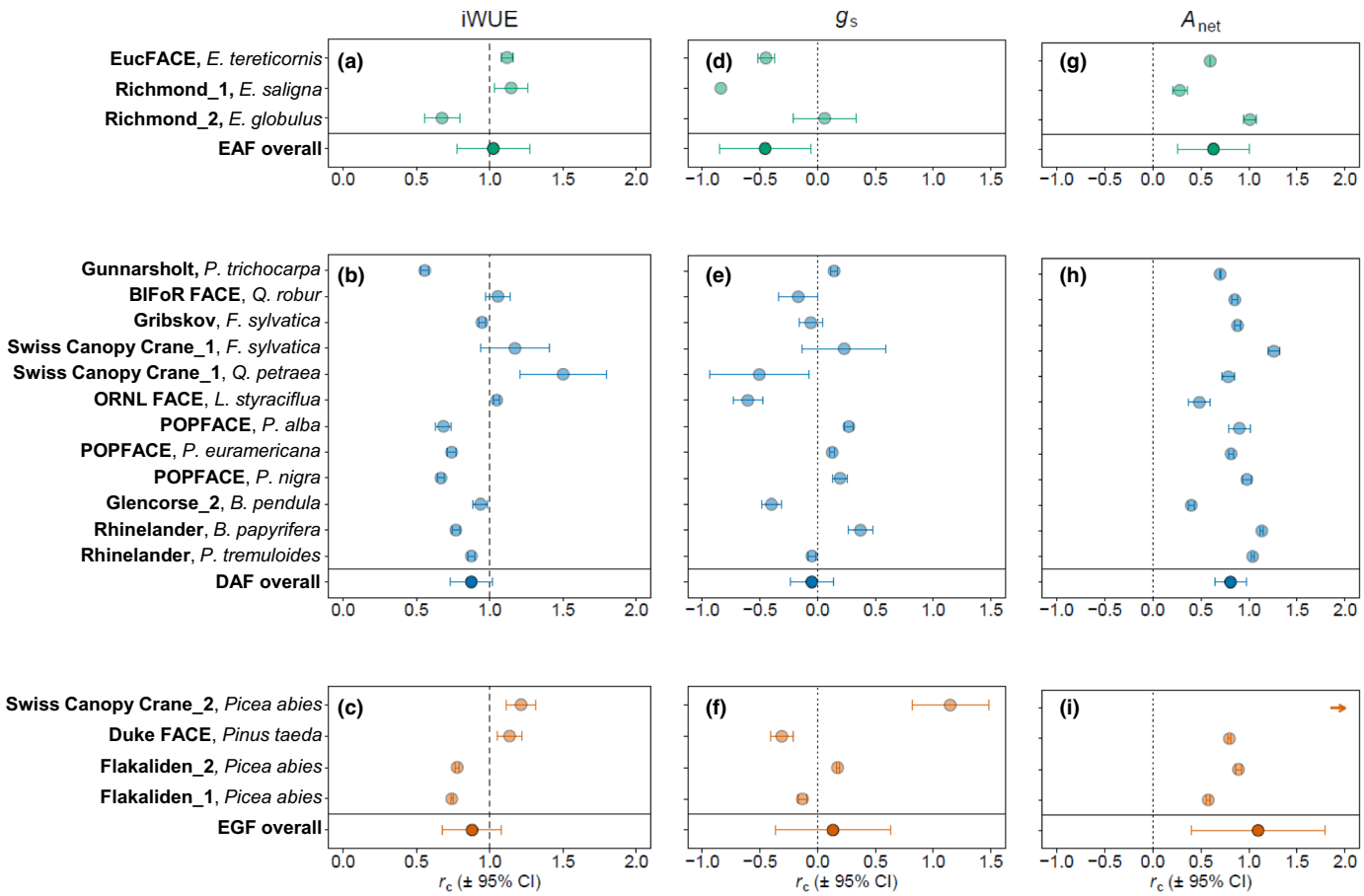

Fig. 1 Estimated effect of eCO_2 on the mean response ratios, that is, a CO_2 normalised response ratio (r_c) of intrinsic water-use efficiency (iWUE; a–c), stomatal conductance (g_s; d–f) and photosynthesis (A_{net}; g–i) across different species and dataset (see Table 1). Each data point represents the mean effect size (overall log response, Eqn 3) of an individual study and species; error bars are the 95% confidence intervals (CI) across the true replicates (n = 2–12). In each plot, the mean (summary) effect size of all the studies for each plant functional type (PFT) is depicted at the bottom. We interpret the CO_2 effect at the 1.0 line (vertical dashed line in a–c) to be in proportion to the CO_2 increase (see main text) and the zero line (vertical dotted line in d–i) represents no significant CO_2 effect (i.e. no response reference line). Arrow in (i) represents data point that extend beyond the limits of the plot. Colours refer to PFT with green as evergreen angiosperm (EAF), blue as deciduous angiosperm (DAF) and orange as evergreen gymnosperm (EGF). The mean response ratios represent, on average, an increase in CO_2 from 383 to 628 μmol mol^{-1}.

Table 3 Between-group heterogeneity for the effect of eCO_2 treatment on iWUE, A_{net} and g_s.

Variable	Q_M	P-value
iWUE	0.77	0.67
g_s	4.24	0.12
A_{net}	1.99	0.37

Plant functional type (PFT; i.e. DAF, EAF and EGF) was used as a moderator in the multivariate linear mixed-effects model. Results were based on log-transformed response ratios. We tested whether plant responses to iWUE, g_s and A_{net} under eCO_2 were different between PFT. Q_M refers to the test statistic for the omnibus test of coefficients of PFT group effect, with $P < 0.05$ indicating significant differences between the PFTs. Response variables are as follows: intrinsic water-use efficiency (iWUE, mol CO_2 mol^{-1} H_2O), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) and photosynthetic rate (A_{net}; μmol m^{-2} s^{-1}) response ratios.

−0.06); Fig. 1g), but neither DAF nor EGF showed significant decreases in g_s with eCO_2 (mean r_c = −0.05 (95% CI: −0.23, 0.14) and 0.13 (95% CI: −0.37, 0.63) respectively; Fig. 1h,i). However, a heterogeneity test (Viechtbauer, 2010) did not indicate a significant difference among PFTs in stomatal responses to eCO_2 (Table 3). As can be seen in Fig. 1, and in the CI ranges reported earlier, there is considerable variability in stomatal response across experiments within PFTs, meaning that there is relatively little power to detect differences among PFTs. Overall, however, this analysis does not confirm the hypothesised pattern of stronger g_s responses in angiosperm species than gymnosperm species.

Across all experiments, photosynthesis increased strongly under eCO_2 (overall response of r_c = 0.82, with 95% CI of 0.64–1.01; $P < 0.001$, Fig. 1g–i). The largest average photosynthetic response to eCO_2 occurred in the EGF (mean r_c = 1.1 (95% CI: 0.39, 1.79)), whereas this response in EAF and DAF was marginally lower (mean r_c = 0.63, (95% CI: 0.26, 1.00) and 0.81, (95% CI: 0.64, 0.97), respectively). Similar to g_s, however, statistical analysis did not indicate significant differences in the photosynthetic response to eCO_2 among PFTs (Table 3).

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Table 4 Statistical output from the meta-analysis of the effect of leaf–air vapour pressure difference, *D*, on the intrinsic water-use efficiency (iWUE, mol CO_2 mol^{-1} H_2O), stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) and photosynthetic rate (A_{net}; μmol m^{-2} s^{-1}) response ratios.

	iWUE (mol CO_2 mol^{-1} H_2O)		g_s (mol H_2O m^{-2} s^{-1})		A_{net} (μmol m^{-2} s^{-1})	
PFT	Slope	*P*-value	Slope	*P*-value	Slope	*P*-value
DAF	0.22	**<0.001**	0.14	0.38	0.41	**<0.001**
EAF	0.22	0.075	0.50	**0.006**	0.72	**<0.001**
EGF	0.13	**0.021**	−0.18	**0.03**	−0.10	0.24

Table shows the slope response to *D* and respective *P*-values among plant functional type (PFT) that are shown in Fig. 2. Significant *P*-values ($P < 0.05$) are shown in bold.

Did *D* affect responses to eCO_2?

Variability across experiments in stomatal responses to eCO_2 may potentially be related to differences in the range of *D* in each experiment (Fig. S2). We tested how responses to eCO_2 vary with *D* (Table 4; Fig. 2). Our expectation was that the response of iWUE to eCO_2 would not vary with *D*, whereas the eCO_2 response of A_{net} would increase, and the eCO_2 response of g_s would decrease, with increasing *D*. Our results differed from these expectations in several ways. Fig. 2a shows the CO_2-induced increase in iWUE generally increased with *D* across all PFTs (Table 4). More specifically, the effect of *D* on the iWUE response to eCO_2 was statistically significant for DAF ($P < 0.001$) and EGF ($P < 0.05$), and only EAF behaved in the way we expected.

We also found that the effect of *D* on the responses of A_{net} and g_s to eCO_2 differed among the three PFTs ($P < 0.05$; Fig. 2b,c; Table 4). For EAF, we found a significant effect of *D* on the g_s response to eCO_2 ($P < 0.05$; i.e. increase in *D* led to a smaller reduction in g_s in response to eCO_2), and a significant effect of *D* on the CO_2 response of A_{net} ($P < 0.001$). These responses cancelled each other out to yield no change in the CO_2 response of iWUE (Fig. 2a). For DAF, we found a significant positive effect of *D* on the CO_2 response of A_{net} ($P < 0.001$) but no effect on the response of g_s, yielding an overall positive eCO_2 effect on iWUE. In contrast, for EGF, the effect of *D* on the CO_2 response of iWUE resulted from a significant effect of *D* on the g_s response to eCO_2 ($P < 0.05$), and no effect on the A_{net} response to CO_2.

Optimal stomatal model

To facilitate the interpretation of these differences among PFTs, we fitted the USO model (Eqn 2) to data from aCO_2 and eCO_2 treatments in all experiments (Figs 3, S3). The fitted model generally provided good explanatory power (median R^2 of 0.70). Fitted values of the g_1 parameter ranged from 1.4 to 7.9 $kPa^{0.5}$. The g_1 parameter was highest in the poplar species *Populus nigra* (7.9 $kPa^{0.5}$ (with 95% CI of 6.3–9.4) and 6.7 $kPa^{0.5}$ (with 95% CI of 5.6–7.9) for eCO_2 and aCO_2 respectively) and lowest in *P. abies* (1.4 $kPa^{0.5}$ (with 95% CI of 1.3–1.49) and 1.45 $kPa^{0.5}$ (with 95% CI of 1.42–1.49) both aCO_2 treatments in Flakaliden_1 and Flakaliden_2 respectively). We found that g_1 was significantly lower in EGF species than in DAF and EAF species ($P < 0.0001$, Fig. 3; Table S1). A lower g_1 value is suggested to lead to a larger response of A_{net}. In support of this, we found that the EGF species had both the lowest g_1 and the highest photosynthetic response to eCO_2.

For 17 of the 20 datasets, there was no significant difference between the g_1 parameters estimated for aCO_2 and eCO_2. However, we found that for all three datasets from boreal sites (*P. abies* trees measured at the Flakaliden site in Northern Sweden and *Populus trichocarpa* measured at the Gunnarsholt site in Iceland), estimated g_1 parameters were significantly higher under eCO_2. In addition, we found that g_1 was significantly higher in species that received supplemental water ($P < 0.001$) and decreased significantly with tree age ($P < 0.05$; Table S1).

Discussion

We quantified the relative responses of iWUE, g_s and A_{net} across a set of manipulative experiments on large trees (Table 1). We found that the mean response of iWUE was approximately proportional to eCO_2, as predicted by the USO model, and this response was similar across the three woody PFTs examined in the study. The results also showed that *D* had a significant effect on these responses to eCO_2 in angiosperms but not in gymnosperms, suggesting differing sensitivities to *D* between these PFTs. The use of the USO model allows these differences to be taken into account. Consistent with previous analyses (Medlyn *et al.*, 2011; Gimeno *et al.*, 2016), we found g_1 was not altered by eCO_2 in most experiments, implying that the optimal stomatal theory is largely successful in predicting the coupling of carbon uptake and water loss in field and forest grown trees under eCO_2. Differences in the response of g_s to CO_2 among PFTs were not statistically significant, in contrast to previous studies, but the tendency for a smaller response in gymnosperm species could be captured in the USO model through the lower g_1 values, which drive a larger A_{net} response.

Does iWUE respond in proportion to eCO_2?

The increases in iWUE with eCO_2 are comparable to those reported in meta-analyses of FACE experimental data in Ainsworth & Long (2005; mean $r_c = 1.2 \pm 0.33$). The results are also consistent with a meta-analysis of tree-ring isotopic data consisting of 422 tree species (Adams *et al.*, 2020), although experimental data of tree-ring isotopic analyses in two FACE experiments suggest much higher iWUE responses (Battipaglia *et al.*, 2013; mean $r_c = 1.4$ and 1.3 for Duke University and Oak Ridge National Laboratory, ORNL, respectively). A general review, compiling several different strands of evidence, concluded that most supported an r_c for iWUE close to one (Walker *et al.*, 2020). We conclude that the proportional increase in iWUE with eCO_2 is a reliable response which supports the

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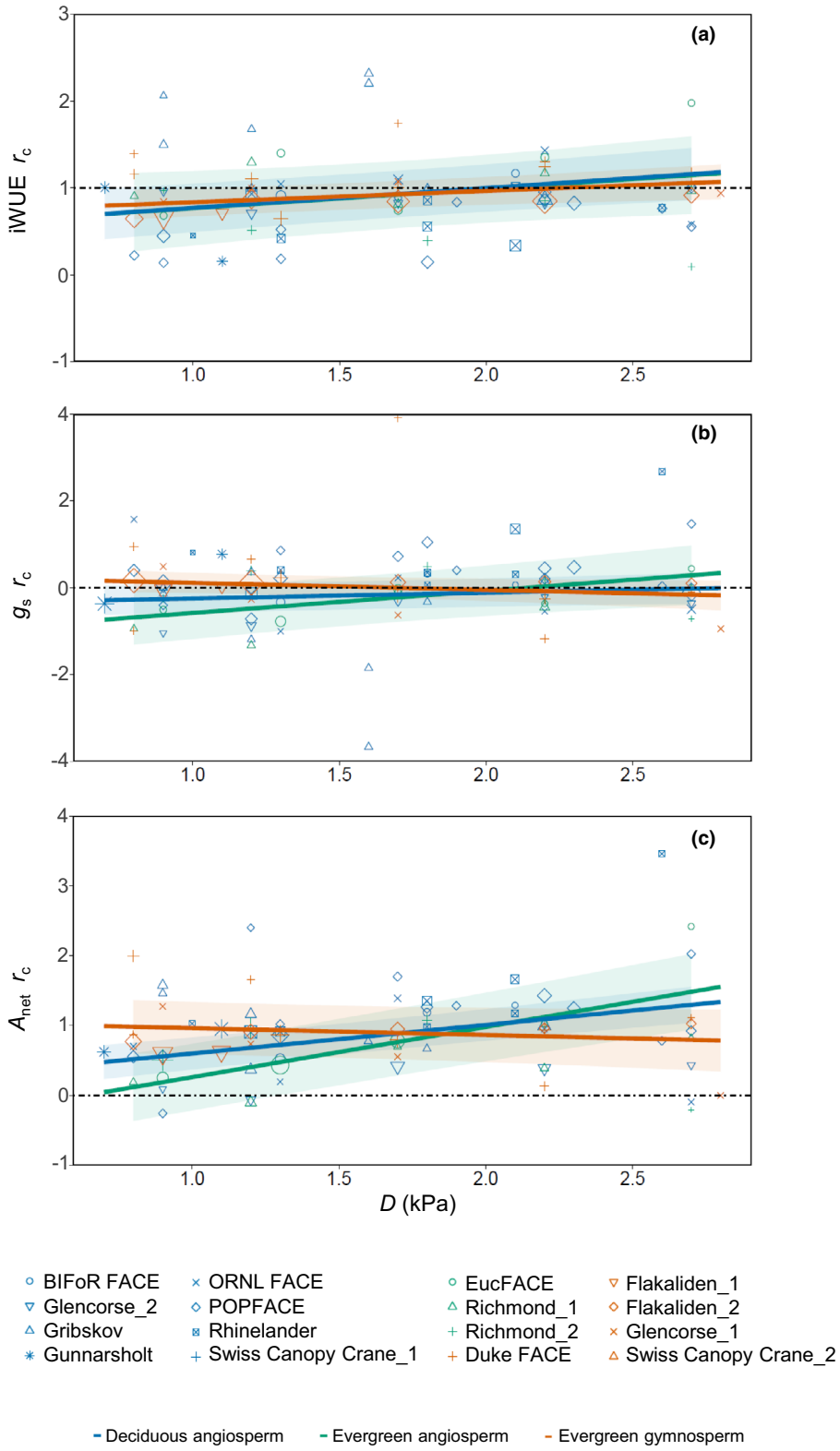

Fig. 2 The effect of *D* on the mean log response ratios for the CO_2 effect (r_c) on (a) intrinsic water-use efficiency (iWUE), (b) stomatal conductance (g_s) and (c) net photosynthesis (A_{net}) across the datasets. Each data point shows the mean response for each 0.5 kPa bin (*D* range of 0.75–2.75) per species per dataset. Symbols refer to dataset and colours refer to plant functional type (PFT) with green as evergreen angiosperm (EAF), blue as deciduous angiosperm (DAF) and orange as evergreen gymnosperm (EGF). Shaded areas are 95% confidence intervals.

optimisation theory that proposes A_{net} and g_s are well coupled to maximise C gain and minimise water loss to the plant (Medlyn *et al.*, 2011; De Kauwe *et al.*, 2013; Walker *et al.*, 2020). This result can therefore allow the prediction of the response of g_s to eCO_2 from the A_{net} response and vice versa.

Which is the primary response variable, A_{net} or g_s?

We found increases in iWUE with eCO_2 were predominantly due to increased A_{net} rather than reductions in g_s. This conclusion is consistent with results from several previous meta-analyses of

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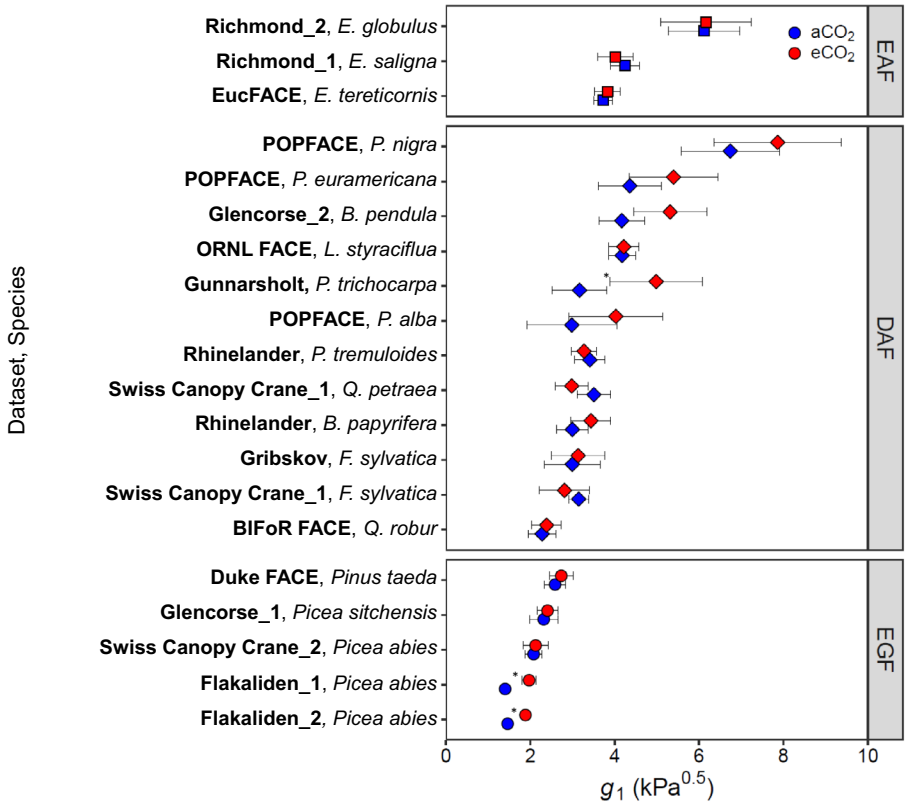

Fig. 3 Fitted parameter with 95% confidence intervals for the unified stomatal optimisation (USO) model (g_1) for each CO_2 treatment per species and dataset, grouped by plant functional type (PFT). Colours indicate CO_2 treatment, with blue for ambient CO_2 (aCO_2) and red for elevated CO_2 (eCO_2). Symbols refer to the PFT with diamonds as deciduous angiosperm (DAF), squares as evergreen angiosperm (EAF) and circles as evergreen gymnosperm (EGF). *, Significant differences in g_1 between CO_2 treatments.

physiological responses of tree species, although the statistical significance of the reduction in g_s varies across studies. Curtis & Wang (1998) found an increase in A_{net} of +54% compared with a non-significant reduction in g_s of −11%. Medlyn *et al.* (2001) found a statistically significant reduction of −14% in g_s compared to an increase in A_{net} of 51% (Medlyn *et al.*, 1999). Similarly, Ainsworth & Rogers (2007) found a statistically significant reduction of −20% in g_s compared with an increase in A_{net} of +45% in tree species. Our results, which include many additional high-quality datasets, show an even stronger influence of A_{net}. We express our findings as a relative response ratio to standardise for the difference in treatment CO_2 concentrations used in different experiments. We find a mean increase (r_c) of 0.82 in A_{net} and no statistical change in g_s (mean reduction of −0.09).

However, we also found the responses of both A_{net} and g_s to eCO_2 can be variable within an experiment, as a result of differences in environmental variables such as D and light. Barton *et al.* (2012) confirmed this finding experimentally at both leaf and whole-canopy scales in *E. saligna* trees and found photosynthetic responses to eCO_2 increased with increases in D. We observed differences in D sensitivity among PFTs for the responses of A_{net} and g_s to eCO_2 (Fig. 2). Changes in A_{net} due to eCO_2 were more responsive to D in angiosperms than in gymnosperms, suggesting a higher sensitivity of physiological processes to D in angiosperms compared with gymnosperms. Our findings show that D needs to be considered when comparing studies where D differs, supporting the use of the USO model to account for differences. It also highlights the limitations on inferences drawn in previous research regarding gymnosperm vs angiosperm responses, as previous studies have generally not accounted for either differences or sensitivity in D when assessing the response of stomata to eCO_2. A further environmental variable that can alter the responses of both A_{net} and g_s is light. A limitation of our study is that we analysed only data with high PPFD (i.e. > 800 PPFD) to allow for comparisons between datasets. Under these conditions, we found strong photosynthetic responses to eCO_2, which may be diminished under lower PPFD (Bernacchi *et al.*, 2003; Uddling *et al.*, 2009; Bader *et al.*, 2016).

To avoid generalisations based on the mean responses of iWUE, A_{net} and g_s due to the effects of differences in D, we recommend using the USO model to normalise iWUE to account for any effect of D. Consistent with the study hypotheses, we found that the optimal stomatal model successfully predicted g_s under both aCO_2 and eCO_2 and the g_1 parameter remained unchanged in response to eCO_2 (Gimeno *et al.*, 2016), with three exceptions (Fig. 3). The three cases where CO_2 treatment significantly affected g_1 were the two *P. abies* datasets at the Flakaliden site (northern Sweden) and *P. trichocarpa* at the Gunnarsholt site (Iceland). The two Flakaliden datasets came from two very different experiments (BB and WTC) carried out a decade apart, suggesting that although the response differs from other experiments, it may be a real effect at the Flakaliden site. The forest soil at Flakaliden is nutrient impoverished (Bergh *et al.*, 1999), so nutrient limitations at the site may have had an effect on leaf physiology at the Flakaliden site and may explain the unusual response observed. This explanation is corroborated by the fact the g_1 parameter remained unchanged with eCO_2 in *P. abies* growing on fertile soil at the Swiss Canopy Crane site

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(Bader *et al.*, 2013). However, other sites also have highly nutrient-limited soils, including the EucFACE experiment. Alternatively, it is notable that all three exceptions were from boreal study sites, suggesting that trees in boreal climates may deviate in responses of stomata to eCO_2. However, we only had three suitable datasets from boreal trees; it would be valuable to test this discrepancy in other boreal sites.

Other limitations of the database available for this study are that we only had one genus within the EAF group, all from one geographical location, and no data at all from the tropics. This motivates future experiments with a focus on a wider range of boreal species in addition to more EAF species, from different regions such as in tropical forests, to confirm the stomatal responsiveness with eCO_2 of these PFTs.

Overall, this study found that the optimal stomatal model was successful in characterising the response to eCO_2 across a wide range of experiments and suggests that the coupling between A_{net} and g_s remained unchanged to eCO_2 Hence, we can recommend that land surface models would benefit from using the g_1 parameter to characterise the behaviour of stomata to a changing environment. However, it is also important to recognise that we limited our dataset to conditions of $PPFD > 800$ μmol and $D < 3$ kPa to maximise comparability among datasets. It is possible that responses to CO_2 will differ at the leaf scale compared to canopy scale (Keenan *et al.*, 2016) as different light and D conditions prevail in the lower canopy (Crous *et al.*, 2020). Further work will be required to assess the model under different conditions, and test scalability to the canopy.

How should we model differences among PFTs?

Previously, research has suggested that physiological responses to eCO_2 differ among woody PFTs, with smaller iWUE and g_s responses observed in gymnosperms than in angiosperms (Saxe *et al.*, 1998; Medlyn *et al.*, 2001; Niinemets *et al.*, 2011; Klein & Ramon, 2019). One goal of this work was to evaluate how this difference among PFTs could be captured in the USO or similar stomatal models. In contrast to previous research, we found that the increases in iWUE with eCO_2 were broadly similar across the three PFTs examined in the study. When examining the analysis of this study with that of previous analyses, the main difference lies in the calculation of the responses. Previous analyses predominantly examined absolute responses to eCO_2, in contrast to relative responses in this study. Therefore, starting stomatal values in previous analyses were generally lower in EGF than in both DAF and EAF species and likely influenced the conclusions (Klein & Ramon, 2019). The strength of this analysis is that we examined the relative responses using replicates, as stated by each individual dataset, as well as including the capacity to correct for D.

We also found no significant differences among PFTs in the eCO_2 responses of g_s (Fig. 1; Table 3). However, we did observe that the g_s response in the evergreen gymnosperms was least sensitive to eCO_2 and g_s was significantly reduced with eCO_2 in evergreen angiosperms. We also found that there was a tendency for EGF species to have a higher A_{net} response. This outcome may be a result of the fact that this PFT also had the lowest g_1 values, consistent with other reviews of species differences in g_1 (Lin *et al.*, 2015). A low g_1 implies a low intercellular CO_2 concentration and thus a higher responsiveness of photosynthesis to rising CO_2. As A_{net} and g_s are coupled in the USO model, this will also result in a smaller responsiveness of g_s.

Conclusion

This study provides a large body of data collected over the last few decades that incorporates a number of tree species and experiments, including recent forest FACE experiments, which is a robust base to evaluate g_s under elevated CO_2. It resolves some of the long-standing questions regarding stomatal behaviour in elevated CO_2 in woody species. The results show that the eCO_2 responses of iWUE, g_s or A_{net} were not significantly different among three key PFTs (Table 3), which has importance to how large-scale models depict these responses (Kleidon, 2004). The results suggest that increases in A_{net}, rather than reductions in g_s, drove the increases in iWUE that occur with eCO_2 in all vegetation types. However, the results highlighted the importance of incorporating environmental factors, such as D, when comparing vegetation type. Our analysis of CO_2 responses suggests that angiosperms were more responsive to D than gymnosperms (Fig. 2; Table 4).

With the exception of data from boreal study sites, the optimal stomatal model was successful in describing the stomatal behaviour with changes in atmospheric variables, such as with eCO_2 and D (Fig. 3). We highlight the areas where data representation needs improvement but conclude that a stomatal parameter such as g_1 can be implemented in ecosystem to land surface models as a reliable way to characterise the key aspects of stomatal behaviour that affect hydrology and land surface energy exchange will remain important as atmospheric CO_2 increases into the future.

Acknowledgements

AG gratefully acknowledges a studentship provided by the John Horseman Trust and the University of Birmingham. The BIFoR FACE facility is supported by the JABBS Foundation, the University of Birmingham and the John Horseman Trust. ARMK acknowledges support from the UK Natural Environment Research Council through grant NE/S015833/1. MJ and BEM acknowledge funding from the Australian Research Council (DE210101654, FL190100003).

Author contributions

AG, BEM and DSE conceived the paper, whilst AG was undertaking doctoral research supervised by ARMK and JP. AG wrote the paper with major contributions from BEM, DSE and MJ. AG, MK-FB, CVMB, CB, CC, DSE, KYC, MED, TEG, MH, SLamba, SLeuzinger, JU, JW and GW collected the data for this manuscript. AG organized the datasets under the supervision of BEM, DSE and MJ. MJ and AG designed and performed the statistical analyses, with input from BEM and DSE. AG and BEM wrote the first draft of the paper. AG, MJ, DSE, RMK, JP,

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MK-FB, CVMB, CB, CC, KYC, MED, TEG, MH, SLamba, SLeuzinger, JU, JW, GW, BEM contributed to the interpretation of the results and intellectual input of this manuscript revision. All authors read and approved the submitted version.

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Data availability

The data that support the findings of this study are publicly available via FigShare (doi: 10.6084/m9.figshare.21252609.v1).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Mean ambient CO_2 (aCO_2; blue) and elevated CO_2 (eCO_2; red) for each of the studies in the dataset.

Fig. S2 Histograms showing the counts of binned D (0.5 kPa) across the ambient CO_2 (blue) and elevated CO_2 (red) treatments for each of the studies in the dataset.

Fig. S3 Relationship between stomatal conductance (g_s) and an index based on the USO formulation calculated from D, A_{net} and CO_2 for each dataset (cf. Medlyn *et al.*, 2011).

Table S1 Between-group heterogeneity for the effect of plant functional type (PFT) (i.e. DAF, EAF and EGF), Tree age (i.e. Old, Mature and Sapling) and Water condition (i.e. Irrigated and Not irrigated) on g_1 ($kPa^{0.5}$) values.

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